

Coil-helix transition in poly(L-glutamic acid) : Evidence for a 3-state non-cooperative process

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A careful analysis of measurements of circular dichroism of poly(L-glutamic acid) (PGA) shows that the data can be very accurately described by introducing a third state for the PGA configuration, in addition to the helix and coil ones, and considering a simple equilibrium between these three states, without cooperativity. The third state is more conspicuous when high molecular weight polyethyleneglycol (PEG) is added. Excluded volume effects shown by differences in presence of short and long PEG chains indicate a direct interaction of PEG and PGA rather than an osmotic effect.

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I. INTRODUCTION

Proteins are important building blocks of life. They have multiple properties due to the interplay of their four structural levels : primary (sequence of aminoacids), secondary (alpha helices, beta sheets, etc.) tertiary (arrangements of secondary structures in space) and quaternary (self assembly of supramolecular structures). The primary structure is fixed by covalent bonds and is therefore very stable. The other levels of order are governed by much weaker interactions (hydrogen bonds, dipolar interactions, etc.) and are therefore sensitive to changes in the environment such as temperature, pH, etc. Understanding the formation of these structures is an important step in the understanding of their overall properties. Moreover it is strongly suspected that misfoldings are responsible for serious diseases [1]. We focus here on the formation of alpha helices from random coils in a much simplified system : the poly(L-glutamic acid) (PGA). The helical parts are optically active and their population can be easily and accurately measured by circular dichroism.

This transition is usually analysed in terms of a theory due to Zimm and Bragg [2, 3] (or variants thereof [4–6]). In this theory an elementary segment of the chain can exist in two states: either as a disordered chain or as a loop. A free energy difference between these states comprises energy and entropy terms :

$$\Delta F = \Delta E - T\Delta S \quad (1)$$

where ΔE and ΔS can be considered as independent of the temperature in a first approach. (A more complete description [7] introduced a linear temperature variation in ΔE and a logarithmic one in ΔS . The non linearity in the log function in the range from 280K to 370K is too small to be seen so that the equation applies with a different meaning of E and S). We choose the energy

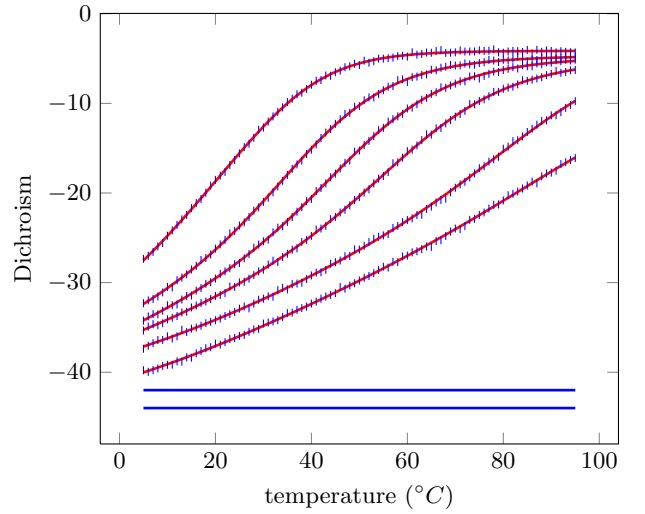


FIG. 1: Measured dichroism (in $degrees.cm^2/mole$) . From top to bottom : 0%, 5%, 7.5%, 10%, 20% and 30% PEG. Red lines are fits using the 3state non-cooperative model. Horizontal lines show the range of possible low-temperature asymptots (see text).

unit such that $k_B = 1$. In the absence of other interactions, the relative ratio of states would be given by a Boltzmann factor $\exp(-\Delta F/T)$. In addition, Zimm and Bragg introduced an interaction between neighbouring elements which facilitates the formation of a new loop beside an existing one and therefore the transition of the whole chain. The problem is therefore similar to the 1D Ising model and can be solved.

Many sets of data have been analysed using this model [6, 8–10] and significant cooperation parameters determined even though a glance at the figures shows a rather poor fit to the data. A new set of measurements using solutions with added polyethyleneglycol (PEG) and at pH=3.75 have been performed using state-of-the-art equipment [11] and will be the basis of our analysis. The results are plotted in figure 1.

Without PEG, the transition occurs at low tempera-

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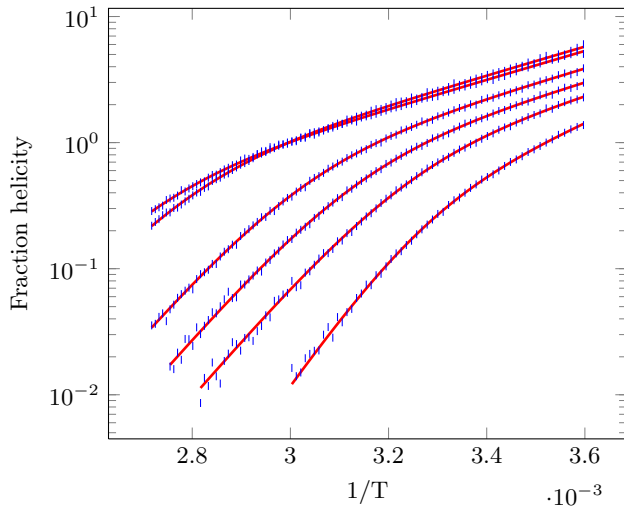


FIG. 2: Same data as in fig 1 expressed as the ratio of optically active peptide to optically inactive ones vs the inverse temperature. From bottom to top: 0%, 5%, 7.5%, 10%, 20% and 30% PEG. Red lines are fits using the 3state non-cooperative model.

ture and only a part of it can be studied. The addition of PEG shifts the transition to higher temperatures but modifies the system. The figure suggests however that these modifications do not change qualitatively the basic mechanism.

II. CRITICAL PLOT OF THE DATA

A more clear picture can be obtained by plotting the data in a different way : Eq1 can be rewritten as :

$$\log[x/(1-x)] = \Delta E/T - \Delta S \quad (2)$$

where x is the fraction of helices. In the absence of cooperativity a plot of $\log(x/(1-x))$ vs $1/T$ would be a straight line and strong cooperativity would lead to a sigmoidal shape.

Computing the helical fraction is, however, not straightforward because it requires the knowledge of the asymptotic values reached by the curves of fig 1 at high and low temperature, corresponding to fractions of helices of 0% and 100% . These are the optical properties of given molecular conformations and should not vary appreciably with temperature. The high temperature limit can be safely assessed for the data with 0% and 5% of PEG . A value of 37.4° has been published [12] for the helix maximum contribution (difference between the upper and lower asymptotes). We allowed for a variation of a few degrees of each of these values to get the most consistent pattern.

We notice first that all the curves of fig 2 have a similar shape: two linear parts at low and high temperature matched by a smooth crossover.

III. THE 3-STATE MODEL

Obviously the data cannot be fitted by any of the shapes predicted by the theory of Zimm and Bragg. However, the presence of two straight asymptotes hints to the occurrence of two transition free energies implying three thermodynamic states. The fraction of helices, in the absence of cooperativity, is easily computed from Boltzmann factors :

$$\frac{x}{(1-x)} = \frac{\exp(-\Delta F_1/T)}{1 + \exp(-\Delta F_2/T)} \quad (3)$$

or

$$\frac{x}{(1-x)} = \frac{\exp(-(\Delta E_1/T - \Delta S_1))}{1 + \exp(-(\Delta E_2/T - \Delta S_2))} \quad (4)$$

taking the coil state as the reference one. (The curvature of the lines implies that only one state is optically active.)

Each curve can be fit very accurately using 6 independent parameters. The values found for the limiting values and the energies are reasonable. However, many of these values cannot be considered as precisely determined. The high helicity side of the PEG poor samples and the low helicity side of the PEG rich samples are not sufficiently present in the experimentally accessible range. Moreover a slight change in a limit value leads to a change in the corresponding energy without altering significantly the quality of the fit. Among the many possible ways of constraining the fit, we chose to explore a possibility suggested by the figures namely constant energies (slopes) for all concentrations. The trial energies are taken from the best fit of the ‘best balanced’ curve at 10% PEG. They are $10400K$ and $7780K$ or $21kCal.M^{-1}$ and $15.5kCal.M^{-1}$ The fit of the experimental data with this formula can be considered as quite satisfactory for all the samples including PEG as can be seen in fig 1 and fig 2. The data without PEG however did exhibit a systematic deviation and a different set of values of energies had to be found . The values are $13500K$ and $8870K$ or $27kCal.M^{-1}$ and $17.8kCal.M^{-1}$. This fit is the one plotted in fig 1 and fig 2. This ‘constant energy’ constraint is only a heuristic and fits with constant entropies and adjustable energies are as good.

IV. EFFECT OF PEG CHAIN LENGTH

The results of a similar study have been published a few years ago[10]. The main difference is that its authors used short PEG chains while Koutsioubas et al. [11] use long PEG chains . These data (picked from the published figure) can be plotted using the same process (figure 3). A look at figure 3 reveals similarities and differences. First the pattern of two transitions matched by a crossover is kept, but the third state is much less conspicuous. We shall therefore not try to perform any quantitative analysis for this state. The energy difference between the coil and helix states (slope of the data at small

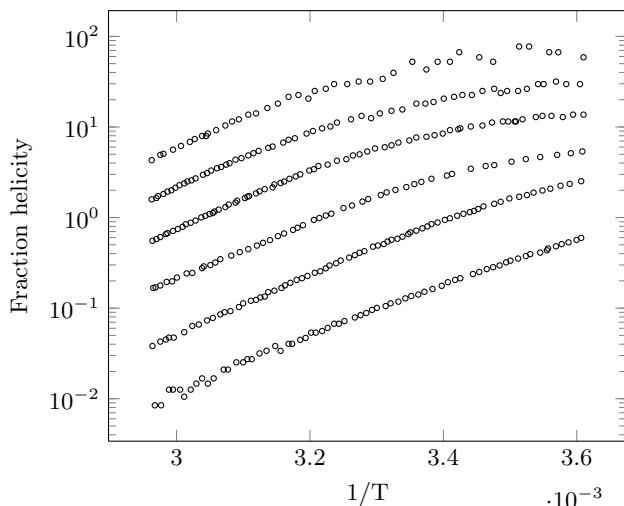


FIG. 3: Ratio of optically active peptide to optically inactive ones from Stanley and Strey[10]. From bottom to top : 10%, 15%, 20%, 25%, 30% and 35 % PEG (of low molecular weight)

$1/T$) is equal to that observed for long chains. A striking feature is that the addition of some short PEG has always a roughly constant effect, while the shift of the data due to adding long PEG clearly levels-off. To illustrate this point we have plotted the temperature $T_1 = E_1/S_1$ vs. the fraction of PEG for all the curves (figure 5). The data cannot be compared one to one because of the different pH but the trend of the curves seems significant. The idea is that a peptide can interact with more and more small molecules when the concentration of these molecules is increased, while the excluded volume effects prevent this for macromolecules. This shift has therefore probably an entropic origin. A mechanism related to osmotic stress, as suggested by Stanley and Strey [10] should depend on the concentration of PEG but not on the length of the chains.

V. FINAL REMARKS

The presence of a third state between coil and helix should not be too surprising. Indeed the amino acids involved are very prone to interacting with each other leading to different structures. A hairpin structure, precursor of a beta sheet, could be a likely hypothesis. A similar study with different lengths of another peptide molecules (AEAAKA) has been published [8, 9]. Our analysis of the data from the published figures shows that the third state is not visible for the shortest chains (up to 26 residues) but seems to appear for the longer

ones (32 or more residues). This clearly favors a third state induced by a self interaction of the chains. The characterisation of the third state by neutron or X-ray scattering can be envisioned but the simultaneous pres-

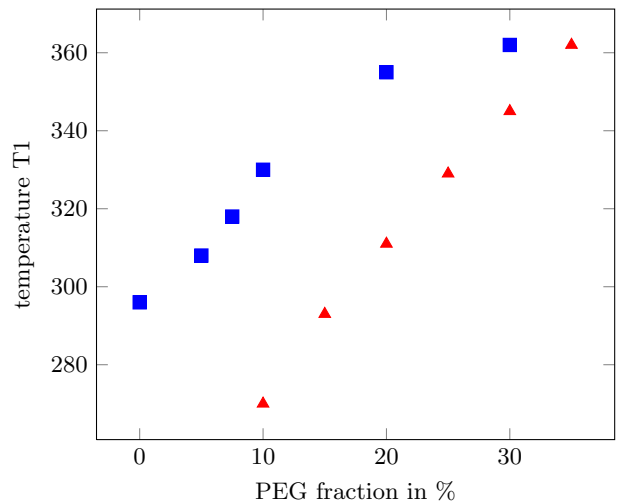


FIG. 4: Temperature T_1 at which the ratio $helix/coil = 1$ vs PEG concentration. Blue squares : long PEG chains, red triangles : short PEG chains

ence of several conformations would make the unraveling of the spectra difficult. A X-ray study by Muroga et al [13] concluded that the observed data did not fit with what was expected for a coil when a coil was expected. Indeed the observed spectra decrease more slowly than expected at large wavevector indicating a more compact state of the scattering objects. A study of the dynamics of the helix-coil transition for PGA also concluded that this process was not a two-state process.[14]

VI. CONCLUSION

The first conclusion of this study is that the sharpening of the transition due to first neighbour interaction as predicted by Zimm and Bragg [2] is nowhere seen. Instead the consideration of three states in mere chemical equilibrium describes accurately the data. The nature of the third state, which may have a significant importance in health related problems remains to be investigated as well as the exact interaction of PGA with PEG. The evidence of excluded volume effects indicates a direct interaction. Circular dichroism measurements for PEG concentrations between 0% and 5% could be useful for this purpose.

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